Passive Smoking Alters Lipid Profiles in Adolescents

Joseph Feldman, DrPH‡; I. Ronald Shenker, MD*; Ruth A. Etzel, MD, PhD§; Francis W. Spierto, PhD§; David E. Lilienfield, MD, MPH, MS, Engin‖; Michael Nussbaum, MD*; and Marc S. Jacobson, MD*

*From the *Division of Adolescent Medicine and Center for Atherosclerosis Prevention, Schneider Children's Hospital of Long Island Jewish Medical Center, the Long Island campus for the Albert Einstein College of Medicine, New Hyde Park, New York; ‡Department of Preventive Medicine, SUNY Health Science Center of Brooklyn, New York; §Center for Environmental Health and Injury Control, Centers for Disease Control, Atlanta, Georgia; and ‖Division of Environmental and Occupational Medicine, Mt Sinai School of Medicine, New York*

ABSTRACT. Although cigarette smoking is associated with elevation of plasma lipid levels and changes in lipoprotein distribution, it is not known whether passive smoking is associated with an alteration in lipid profiles. The relation between plasma cotinine, a marker of exposure to tobacco smoke, and lipid profiles was studied in healthy adolescents from a suburban New York high school district who were undergoing preparticipation sports physicals. Forty-four percent of the adolescents reported that one or both parents currently smoked. Eleven percent of the adolescents had plasma cotinine concentrations ≥2.5 ng/mL, the level considered indicative of exposure. Adolescents with two smoking parents had significantly higher plasma cotinine concentrations after adjustment for other factors than adolescents whose parents did not smoke. Plasma cotinine concentration ≥2.5 ng/mL was associated with an 8.9% greater ratio of total cholesterol to high-density lipoprotein cholesterol ($P < .003$) and a 6.8% lower high-density lipoprotein cholesterol ($P < .03$). These results suggest that passive smoking, like active smoking, leads to alterations in lipid profiles predictive of an increased risk of atherosclerosis. *Pediatrics* 1991;88:259–264; *passive smoking, adolescents, cotinine, lipid profiles, cholesterol.*

ABBREVIATIONS. TOTAL-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; CV, coefficient of variation; BMI, body mass index; CI, confidence interval.

Received for publication Jul 9, 1990; accepted Oct 20, 1990.
Reprint requests to (M.S.J.) Center for Atherosclerosis Prevention, Schneider Children's Hospital, Room 187, Long Island Jewish Medical Center, New Hyde Park, NY 11042.

Cigarette smoking is associated with elevation of plasma lipid levels and changes in lipoprotein distribution,[1] including an elevated ratio of total cholesterol (TOTAL-C) to high-density lipoprotein cholesterol (HDL-C).[2–4] The TOTAL-C/HDL-C ratio is a powerful predictor of the risk of atherosclerotic cardiovascular disease and therefore its relationship to passive as well as active smoking has implications for pediatric atherosclerosis prevention.[5,6]

The present study investigated the relationship of passive smoking to lipid profiles in healthy adolescents. Cotinine, a major metabolite of nicotine, was used as a marker of passive exposure to tobacco smoke.[7,8] We hypothesized that passive exposure to environmental tobacco smoke as indicated by plasma cotinine concentration would be associated with an increase in the TOTAL-C/HDL-C ratio.

METHODS

As part of a required health risk assessment and preparticipation sports physical examination, nonfasting whole blood samples were obtained from 444 students attending suburban New York high schools in August 1987. All students trying out for an athletic team at the high schools took the physical. Students were asked to complete self-administered questionnaires about their cigarette smoking habits and diet. This questionnaire has previously been found to be reliable.[9] In addition, students were interviewed by one of two authors (J.F., M.S.J.) regarding cigarette smoking habits of

their parents, siblings, and friends. To improve reliability, the first 10 interviews conducted by each interviewer were observed by the other interviewer. The procedures followed were approved by the Human Subjects Review Committee at Long Island Jewish Medical Center.

Passive exposure of tobacco smoke was grouped into five mutually exclusive categories based on the current cigarette smoking habits of the students' parents, siblings, and friends. The first three groups below were based solely on exposure to parental smoking without regard to sibs or friends. The five categories were as follows: (1) mother smoked but father did not, (2) father smoked but mother did not, (30 both parents smoked, (4) siblings and/or friends only smoked, and (5) no parents, siblings, or friends smoked. Exposure from any parents not currently living with the subject was excluded. Exposure from smoking friends was excluded if the student reported spending ≤2 hours per week in their company. Nonfasting plasma was collected by venipuncture from seated subjects, centrifuged, and frozen until analysis.

Cholesterol and triglycerides were assayed directly from 10 μL of supernatant by a Kodak multilayer film method. High-density lipoprotein cholesterol was determined by drawing 0.5 mL of plasma into tubes containing 50 000 molecular weight dextran sulfate-magnesium reagent and centrifuging the mixture at 1500 × g for 10 minutes to precipitate the very-low-density lipoprotein and low-density lipoprotein particles. The HDL-containing supernatant was then assayed directly for cholesterol using the Kodak multilayer film method. We analyzed 15 paired samples, both on a Kodak DT60 Analyzer and at Queens Hospital Center (New York) Arteriosclerosis Research Laboratory, which is a participant in the Centers for Disease Control/National Heart, Lung, and Blood Institute Lipid Standardization Program. Correlation coefficients were cholesterol = .99, triglycerides = .97, and HDL-C = .91.

Total cholesterol and HDL-C were measured in 11 batches. The coefficient of variation (CV) of total cholesterol by batch ranged from 0.16 to 0.20 whereas for HDL-C the CV ranged from 0.16 to 0.27. There were no significant differences in the average levels or the variation from the averages among the 11 batches in either males or females. Four hundred twenty-five of the students had sufficient plasma remaining for cotinine analysis. Plasma cotinine analysis was performed at the Division of Environmental Health Laboratory Sciences at the Centers for Disease Control using the radioimmunoassay previously described by Knight et al.[10] The level of detection for cotinine in this assay was 1.6 ng/mL. Cotinine and lipid concentrations were each determined without knowledge of reported exposure to tobacco smoke.

Notched box plots in the Figure were used to indicate the median cholesterol concentrations and the 95% confidence interval about the medians. If the notches in the boxes (ie, median ± 1.57 × interquartile range/$\sqrt{n}$) do not overlap, this can be regarded as strong evidence that the population medians differ.[11-13] Hatched areas in the Figure denote the area between the 75th and 25th percentile (ie, interquartile range).

Multiple linear analyses of covariance were performed using TOTAL-C, HDL-C, and ratio of TOTAL-C/HDL-C as outcomes. Covariates included age, race, sex, triglyceride concentration, and body mass index (BMI). Body mass index was calculated by dividing weight by the square of height. The predictor variables were plasma cotinine concentrations and self-reported passive tobacco smoke exposure. Inasmuch as the distributions of both cotinine and triglyceride concentrations were highly skewed, these data were logarithmically transformed. This transformation of the serum cotinine levels was also useful in testing for differences among the exposure groups, inasmuch as the similarity of the CV of cotinine among the exposure categories (from 1.6 to 2.4) suggested proportional effects.[14] We report both the arithmetic and geometric means of cotinine. The constant 0.05 was added to all cotinine concentrations to avoid the logarithm of zero which is undefined. In addition,

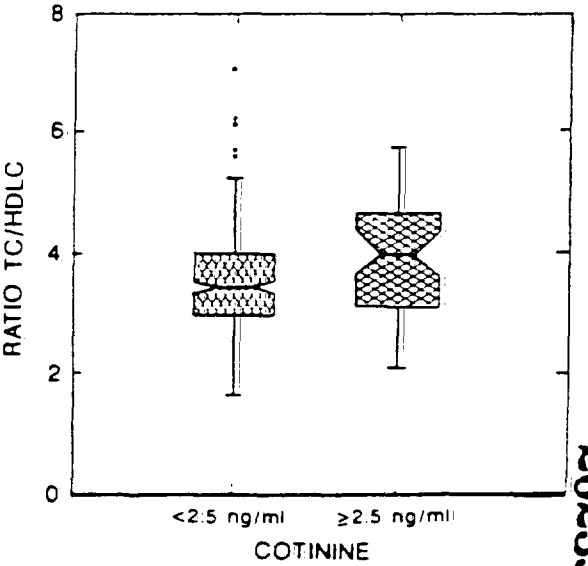

Figure. Lipid ratio in passive smoke-exposed and nonexposed adolescents. TC, total cholesterol; HDLC, high-density lipoprotein cholesterol; *, outlier values.

plasma cotinine concentrations were categorized as <2.5 ng/mL or ≥2.5 ng/mL to indicate exposure based on previous work.[15] Interactions between the covariates and the predictor variables were examined and none was significant. The adequacy of the assumptions underlying the various models was assessed by examining various residual plots.[11] Reanalysis omitting highly leveraged cases (ie, dropping cases with a much greater than average impact on results) did not change any conclusions. The actual computations were performed using Systat.[16]

Self-reported smokers (n = 7) were excluded. To reduce the possibility of including current smokers who did not report accurately, students with plasma cotinine concentrations of more than 25 ng/mL (n = 12) and nonresponders to the smoking question (n = 2) were also excluded.[7] In addition, we excluded 7 adolescents on cholesterol-lowering diets and 6 nonresponders to the diet question. The study sample therefore consisted of 391 adolescents. The analyses in Tables 3 and 4 were also done excluding 5 adolescents with serum cotinine values of 11 to 25 to further reduce the possibility of misclassification of active smokers. The results were almost identical and therefore are not shown.

RESULTS

The sample included 274 boys (69.7%) and 117 girls (30.3%). Two hundred seventy-eight (71.1%) were white, 52 (13.2%) were black, 20 (5.1%) were other races, and 41 (10.5%) did not indicate their race. The mean age was 14.8 ± 1.6 years; 34.3% of the adolescents reported no smokers among their parents, siblings, or friends; 15.1% reported that mother smoked but father did not; 17.4% reported that father smoked but mother did not; 11.5% reported that both parents smoked; and 21.7% reported that siblings and/or friends only smoked. The arithmetic mean cotinine concentration was 1.39 ng/mL (SD = 4.70), which was not significantly different from the level of detection of the assay. Eleven percent (n = 44) of the adolescents had plasma cotinine concentrations ≥2.5 ng/mL, 89% (n = 347) had plasma cotinine concentrations <2.5 ng/mL. Both the geometric and arithmetic mean plasma cotinine concentrations were significantly higher among adolescents who reported that one or both parents smoked; the highest level was found among adolescents with two smoking parents (Table 1). Table 2 shows average levels of the ratio of TOTAL-C/HDL-C by reported exposure and category of serum cotinine concentration. The TOTAL-C/HDL-C ratio was always higher in children whose serum cotinine level was ≥2.5 ng/mL irrespective of reported exposure. Despite the statistically significant association between reported exposure and serum cotinine concentration, it was apparent that there was considerable misclassification of exposure based on self-report. Fewer than 20% of the students in any reported exposure category were classified as exposed based on serum cotinine levels greater than or equal to 2.5 ng/mL (Table 2).

Mean TOTAL-C concentration was 154 mg/dL (SD = 27.2), mean HDL-C concentration was 44.6 mg/dL (SD = 10.0), and mean TOTAL-C/HDL-C ratio was 3.58 (SD = 1.86). The Figure shows notched box plots for the ratio of TOTAL-CHDL-C by cotinine group with 95% confidence intervals about the medians. The asterisks in the Figure indicate observations that fall outside the 95% range of individual values. The median TOTAL-C/HDL-C ratio for the 44 adolescents with cotinine concentrations ≥2.5 ng/mL was significantly higher than that for the 347 adolescents with cotinine concentrations <2.5 ng/mL ($P < .002$). This was not true for HDL-C concentrations until covariates were taken into account as described later.

In the analysis of covariance model with outcome equal to the ratio of TOTAL-C/HDL-C, the independent variables BMI, log triglyceride level, and log cotinine level were all significantly associated with the ratio (not shown). Together the variables accounted for 28% of the variation in the ratio of TOTAL-C/HDL-C, with triglyceride concentration and BMI accounting for 97% of that amount. Results of analysis with cotinine grouped into two categories are shown in Table 3. Cotinine level was significantly associated with the ratio of TOTAL-C/HDL-C ($P < .003$). [The regression equation was ratio = −.60 + .038 (BMI) + .771 ln (triglyceride) − .324 (if cotinine level <2.5 ng/mL) + .379 (if white).] For the group with cotinine levels ≥2.5 ng/mL, the ratio of TOTAL-C/HDL-C on average was .324 or 8.9% (95% confidence interval) [CI] 6.9% to 11.0%) higher than if the cotinine level was <2.5 ng/mL. Cotinine level was significantly associated with lower HDL-C concentration ($P < .03$) (Table 4). [HDL-C = 77.8 − 5.4 ln (triglyceride) − 0.48 (BMI) + 3.0 (if cotinine level <2.5 ng/mL) − 2.56 (if male).] The HDL-C level in adolescents with plasma cotinine concentration ≥2.5 ng/mL was 3.0 mg/dL or 6.8% (95% CI 4.6% to 8.9%) lower than in those with plasma cotinine concentration <2.5 ng/mL after adjustment for other factors.

The association of serum cotinine concentration with the ratio of TOTAL-C/HDL-C was examined separately for whites with similar results ($P = .001$), as well as for boys ($P = .014$) and girls ($P = .001$).

TABLE 1. Arithmetic and Geometric Mean Levels and 95% Confidence Intervals (CI) for Serum Cotinine Levels by Reported Exposure

Reported Exposure	n	Arithmetic Mean (95% CI)	Geometric Mean (95% CI)
None	134	0.55 (0.40–0.69)	0.07 (0.04–0.11)
Friends/sibs only	87	1.35 (0.66–2.03)	0.07 (0.04–0.14)
Mother, not father	59	1.06 (0.49–1.62)	0.13 (0.07–0.26)
Father, not mother	66	1.39 (0.75–2.02)	0.21 (0.11–0.39)
Both mother and father	45	2.15 (0.81–3.49)	0.35 (0.17–0.74)
F ratio 4.386 *df*		3.65	4.48
P value		0.006	0.001

TABLE 2. Ratio of Total Cholesterol to High-Density Lipoprotein Cholesterol by Serum Cotinine Group and Reported Exposure

Reported Exposure	Serum Cotinine Concentration					
	<2.5 ng/mL			≥2.5 ng/mL		
	n	Ratio	SD	n	Ratio	SD
None	124	3.47	0.87	10	3.77	0.76
Friends/sibs only	71	3.55	0.90	16	3.70	1.13
Mother, not father	49	3.34	0.55	10	4.06	1.02
Father, not mother	33	3.64	0.78	13	4.22	1.04
Both mother and father	37	3.68	0.85	8	3.91	1.02

TABLE 3. Multiple Regression Analysis of Ratio of Total Cholesterol to High-Density Lipoprotein Cholesterol by Plasma Cotinine Concentration, Grouped, Adjusted for Several Covariates*

Source of Variation	SS	*df*	F Ratio	*P* Value
Race	1.72	2	1.81	.165
Body mass index	6.64	1	13.99	.000
Cotinine grouped: <2.5 ng/mL vs ≥2.5 ng/mL	4.27	1	9.01	.003
Log triglyceride	44.00	1	92.79	.000
Error	160.29	338		

* SS = sum of squares; N = 344; r = .54. Forty-seven patients with missing data on any of the above variables are excluded.

TABLE 4. Multiple Regression Analysis of High-Density Lipoprotein Cholesterol by Grouped Plasma Cotinine Levels Adjusted for Several Covariates*

Source of Variation	SS	*df*	F Ratio	*P* Value
Sex	515.20	1	5.77	.017
Log triglyceride	2346.87	1	26.27	.000
Body mass index	1152.40	1	12.90	.000
Cotinine grouped: <2.5 ng/mL or ≥2.5 ng/mL	427.50	1	4.79	.030
Error	34041.56	381		

* N = 386; r = .35. Five patients with data missing on any of these variables are excluded.

The relationship between reported smoking habits of parents, siblings, and friends and the ratio of TOTAL-C/HDL-C was not statistically significant (P = .18). There was a significant difference in the ratio of TOTAL-C/HDL-C of adolescents whose fathers smoked compared with others ($P \leq .04$). When adjusted for multiple comparison bias, this finding was no longer statistically significant. There was no difference in the ratio of TOTAL-C/HDL-C of adolescents whose mothers smoked compared with others.

DISCUSSION

In this sample, passive exposure to tobacco smoke as indicated by plasma cotinine concentration was associated with a higher ratio of TOTAL-C/HDL-C and with a lower HDL-C concentration.

When other factors were adjusted, passive exposure to tobacco smoke was associated with an increased ratio of TOTAL-C/HDL-C and decreased HDL-C concentration of between 7% and 9%. However, compared with other factors such as BMI or triglyceride concentration, the impact of passive smoking on the ratio of TOTAL-C/HDL-C was relatively small.

We did not measure socioeconomic status of subjects or obtain detailed dietary histories and therefore could not control for these variables. It is possible that parents of lower economic status smoked more frequently and provided their children diets higher in cholesterol and saturated fats, resulting in a secondary association between serum cotinine concentration and lipid ratios. This seems an unlikely explanation, however, because the students came from a relatively homogeneous community.

The association of lipid profiles with reported smoking habits of parents, siblings, and friends was not statistically significant. There was an association for adolescents whose fathers smoked compared with others. But there was no association with mothers' smoking. We did not predict this pattern initially and the observation is inconsistent with other reports, which have found a stronger association with mothers' smoking.[16] The most likely explanation for this pattern is a spurious association. Parenthetically, this pattern also diminishes the likelihood that the observed association between the lipid ratio and cotinine concentration was due to smoking mothers' providing a more atherogenic diet. The association of passive exposure to tobacco smoke with reduced HDL-C and elevated ratio of TOTAL-C/HDL-C is biologically plausible, inasmuch as several investigators have found that cigarette smoking results in a lowering of HDL-C.[1,5,18-20] In one longitudinal study of 36 female volunteers, investigators found that HDL-C levels fluctuated with smoking status, increasing when smoking ceased and decreasing when smoking resumed.[21] In another study, investigators reported a dose-response relationship between smoking and ratio of TOTAL-C/HDL-C.[4] The age- and weight-adjusted ratio of TOTAL-C/HDL-C among 233 randomly selected families was 13% higher for smokers than for nonsmokers, about 1.5 times that seen in this study.

Several investigators have found suggestive evidence of an increased risk of coronary heart disease mortality among adults passively exposed to tobacco smoke. Helsing et al[22] found that death rates from atherosclerotic heart disease were 24% to 31% higher for nonsmokers living with smokers compared with those living with nonsmokers. In a study of nonsmoking women 50 to 79 years old in southern California, those whose husbands smoked had a 10-year mortality from ischemic heart disease that was 2.7 times higher than those whose husbands never smoked ($P \leq .10$).[23,24] In the Multiple Risk Factor Intervention Trial, the effect of exposure to tobacco smoke was assessed among 1245 married men aged 35 to 57 years.[25] The relative risk for nonsmoking men with smoking wives compared with those with nonsmoking wives was 2.1 for coronary heart disease death ($P = .19$); and 1.48 for fatal or nonfatal coronary heart disease events ($P = .13$).

A recent study in 216 families of preadolescent children from the Medical College of Virginia twin study found that children in the 105 families of smoking parents had significantly lower HDL-C and higher whole blood 2,3-*d*-phosphoglycerate levels than children in the 111 nonsmoking families.[26] The authors concluded that children with long-term exposure to passive smoke may be at elevated risk for the development of premature coronary heart disease.

The effect of tobacco on lipid levels provides one plausible mechanism (among others such as platelet aggregation, vasoactivity, and compromised oxygen transport) for the well-established elevation of coronary heart disease risk among smokers and suggests a mechanism for the possible increased coronary heart disease risk in passive smokers.

ACKNOWLEDGMENTS

Partial support was received from National Institute of Environmental Health Sciences grants K08-E500161 and P30-E500928.

We thank Paul Samuel, MD, S. Bader, F. H. Gardner, and L. Markowski for their assistance with various parts of this study and B. North for typing the manuscript.

REFERENCES

1. US Dept of Health and Human Services. *The Health Consequences of Smoking: Cardiovascular Disease. A Report of the Surgeon General.* Rockville, MD: US Public Health Service; 1983. DHHS publication (PHS) 84-50204
2. Criqui MH, Wallace RS, Weiss G, et al. Cigarette smoking and plasma high density lipoprotein cholesterol. *Circulation* 1980;62(suppl):IV-70-6
3. Brischetto CS, Connor WE, Connor SL, Matarazzo JG. Plasma lipid and lipoprotein profiles of cigarette smokers from randomly selected families: enhancement of hyperlipidemia and depression of high density lipoprotein. *Am J Cardiol* 1983;52:675-680
4. Freedman DS, Srinivasan SR, Shear CL, et al. Cigarette smoking initiation and longitudinal changes in serum lipids and lipoproteins in early adulthood: the Bogalusa Heart Study. *Am J Epidemiol* 1986;124:207-219
5. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TI. High density lipoprotein as a protective factor against coronary heart disease. *Am J Med* 1977;62:707-714

6. Wallace RB, Anderson RA. Blood lipids, lipid related measures and the risk of atherosclerotic cardiovascular disease. *Epidemiol Rec.* 1987;9:95–119
7. Jarvis JJ, Pedoe-Tunstall H, Feyerabend C, Vessey C, Saloojee Y. Comparison of tests used to distinguish smokers from nonsmokers. *Am J Public Health.* 1987;77:1435–1438
8. Haley NJ, Axelrod CM, Tilton WA. Validation of self-reported smoking behavior: biochemical analysis of cotinine and thiocyanate. *Am J Public Health.* 1983;73:1204–1207
9. Tsimovianis GV, Jacobson MS, Feldman JG, Antonio-Santiago MT, Clutario BC, Nussbaum M, Shenker IR. Reduction in pulmonary function and increased frequency of cough associated with passive smoking in teenage athletes. *Pediatrics.* 1987;80:32–36
10. Knight GJ, Wilie P, Holman MS, Haddow JE. Improved 125 I radioimmunoassay for cotinine by selective removal of bridge antibodies. *Clin Chem.* 1985;31:118–121
11. Chambers J, Cleveland W, Kleiner B, Tukey P. *Graphical Methods for Data Analysis.* Pacific Grove, CA: Wadsworth Publishing Co; 1984
12. Williamson DF, Kendrick JS. The box plot: a simple visual method to interpret data. *Ann Intern Med.* 1989;110:916–921
13. McGill R, Tukey JW, Larsen WA. Variations of box plots. *Am Statistician.* 1978;32:12–16
14. Snedecor G, Cochran W. *Statistical Methods.* 6th ed. Ames, IA: Iowa State University Press; 1967:329–330
15. Etzel RA. *A Cohort Study of Passive Smoking and Middle Ear Effusions in Children.* Chapel Hill, NC: University of North Carolina; 1985. Dissertation
16. Wilkinson L. *Systat: The System for Statistics.* Evanston, IL: SYSTAT; 1988
17. US Dept of Health and Human Services. *The Health Consequences of Involuntary Smoking: A Report of the Surgeon General.* Washington, DC: US Government Printing Office; 1986. DHHS publication (CDC) 87-8398
18. Castelli WP, Garrison RJ, Wilson PWF, et al. Incidence of coronary heart disease and lipoprotein cholesterol levels: the Framingham Study. *JAMA.* 1986;256:2835–2838
19. Orchard TJ, Rodgers M, Hedley AJ, Mitachell JRA. Serum lipids in a teenage population: geographic, seasonal and familial factors. *Int J Epidemiol.* 1981;10:161–170
20. Halfon ST, Green MS, Heiss G. Smoking status and lipid levels in adults of different ethnic origins: the Jerusalem Lipid Research Clinic Program. *Int J Epidemiol.* 1984;13:177–183.
21. Moffatt RJ. Effects of cessation of smoking on serum lipids and high density lipoprotein cholesterol. *Atherosclerosis.* 1988;74:85–89
22. Helsing KJ, Sandler DP, Comstock GW, Chee E. Heart disease mortality in nonsmokers living with smokers. *Am J Epidemiol.* 1988;127:915–922
23. Garland C, Barrett-Connor E, Suarez L, Criqui MH, Wingard DL. Effects of passive smoking on ischemic heart disease mortality of nonsmokers: a prospective study. *Am J Epidemiol.* 1985;121:645–650
24. Erratum. *Am J Epidemiol.* 1985;122:1112
25. Svendsen KH, Kuller LH, Martin MJ, Ockene JK. Effects of passive smoking in the Multiple Risk Factor Intervention Trial. *Am J Epidemiol.* 1987;126:783–795
26. Moskowitz WB, Mosteller MM, Schieken RM, et al. Lipoprotein and oxygen transport alterations in passive smoking: preadolescent children: the MCV twin study. *Circulation* 1990;81:586–592

PATIENTS' GRADES HELP TO SET PAY FOR HEALTH-PLAN DOCTORS

A growing number of large health-care plans are asking patients to grade their doctors: How long are they kept waiting in the office? Can the doctor be reached at night and on weekends? Does the doctor listen as patients describe symptoms? How well is a treatment explained?

Some health maintenance organizations, or H.M.O.'s, use the grades as one criterion in paying the doctors. Not surprisingly, many doctors think this is a bad idea.

At least 34 million Americans are enrolled in H.M.O. plans, and more than 2.9 million are in plans that use patient evaluations to help determine doctors' bonuses. The number of such plans is steadily increasing.

Freudenheim M. Patients' grades help to set pay for health-plan doctors. *The New York Times.* May 26, 1990.

NOTED BY J.F.L., MD